

# Low folate status is associated with impaired cognitive function and dementia in the Sacramento Area Latino Study on Aging<sup>1-3</sup>

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## ABSTRACT

**Background:** Low folate status is associated with poor cognitive function and dementia in the elderly. Since 1998, grain products in the United States have been fortified with folic acid, which has reduced the prevalence of folate deficiency and hyperhomocysteinemia.

**Objective:** We investigated whether folate status is associated with cognitive function and dementia in a cohort of elderly Latinos (aged  $\geq 60$  y;  $n = 1789$ ) exposed to folic acid fortification.

**Design:** Global cognitive function was assessed by the Modified Mini-Mental State Examination (3MSE) and specific cognitive functions by cross-culturally validated neuropsychological tests. Dementia was diagnosed according to the *American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders, 3rd edition revised*, and California Alzheimer Disease Diagnostic and Treatment criteria. Red blood cell (RBC) folate was measured by automated chemiluminescence and total plasma homocysteine by HPLC.

**Results:** The prevalence of folate deficiency (RBC folate  $\leq 160$  ng/mL) was  $<1\%$ . After control for confounding by homocysteine, vitamin B-12, creatinine, demographic variables, and depressive symptom score, RBC folate was directly associated with 3MSE ( $P = 0.005$ ) and delayed recall ( $P = 0.007$ ) scores. In addition, adjusted odds ratios for low 3MSE score ( $\leq 78$ ) and dementia diagnosis per unit increase in RBC folate were significantly below unity ( $P \leq 0.008$ ), which indicated that the relative risks of cognitive impairment and dementia decreased with increasing RBC folate concentration. In contrast, adjusted odds ratios for low 3MSE score and dementia diagnosis per unit increase in homocysteine were not significant.

**Conclusion:** RBC folate is directly associated with cognitive function scores and is inversely associated with dementia in elderly Latinos despite folic acid fortification. *Am J Clin Nutr* 2005;82:1346–52.

**KEY WORDS** Folate, homocysteine, cognitive function, dementia, Modified Mini-Mental State Examination, vitamin B-12, creatinine, Center for Epidemiologic Studies Depression scale, elderly, aging, Latinos

## INTRODUCTION

For more than a decade, elevated plasma concentration of the sulfur amino acid homocysteine (hyperhomocysteinemia) has been recognized as an independent risk factor for cardiovascular, peripheral vascular, and cerebrovascular disease (1). Moreover, hyperhomocysteinemia is associated with Alzheimer disease,

dementia, and indexes of cognitive function in case-control studies of patients with psychogeriatric conditions (2–5) and in cross-sectional, population-based studies of community-dwelling older adults (6–9). In our own investigation of elderly Latinos (10), we observed modest inverse associations between homocysteine and the global Modified Mini-Mental State Examination (3MSE) assessment, and on several tests of cognitive subdomains, including picture association, verbal attention span, and pattern recognition.

The role of folate in homocysteine metabolism is fundamentally important. Folate is required for the biochemical conversion of homocysteine to methionine and the subsequent synthesis of S-adenosylmethionine (SAM). S-Adenosylhomocysteine, a product of SAM-dependent methylation reactions, subsequently loses its adenosyl group to form homocysteine. Homocysteine can then enter a new cycle of methionine synthesis and methylation, or it can be catabolized through cystathionine synthesis. Elevated plasma homocysteine is one of the primary consequences of folate deficiency. Furthermore, older people with low folate status are at a higher risk of cognitive impairment, dementia, and Alzheimer disease (6, 11–16), and it has been postulated that the effect of folate deficiency on brain function is mediated by homocysteine. However, several studies found that the association between low folate status and cognitive impairment, dementia, or Alzheimer disease remains significant after controlling for confounding by homocysteine, thus suggesting that folate may affect brain function through mechanisms not directly related to hyperhomocysteinemia (6, 11–16).

In January 1998, the US government mandated the fortification of grains (eg, cereals, breads) with folic acid to lower the incidence of neural tube birth defects (eg, spina bifida,

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anencephaly). The program of folic acid fortification has been successful in reducing both the prevalence of folate deficiency and hyperhomocysteinemia in the general population (17, 18) and the incidence of neural tube birth defects (19). Because of its success, we investigated whether folate status is associated with cognitive function scores and dementia diagnosis in a cohort of older adults exposed to folic acid fortification. We addressed this issue in a cross-sectional analysis of data from the Sacramento Area Latino Study on Aging (SALSA), a community-based cohort study of physical and cognitive functioning in elderly Latinos (age  $\geq 60$  y).

## SUBJECTS AND METHODS

### Subjects

A cohort of community-dwelling elderly Latinos ( $n = 1789$ ; age  $\geq 60$  y), residing in Sacramento, CA, and surrounding northern California communities, were recruited during a period of 1.5 y, beginning in February 1998 (ie, after the initiation of folic acid fortification). Subjects were considered "Latino" if they, their parents, or their grandparents were born in Mexico, Central America, or South America. The details of sampling and recruitment are described elsewhere (20). The study was approved by the University of California Davis Institutional Review Board, and informed consent was obtained from all subjects.

### Sample collection and analyses

Fasting blood was collected from each participant during home visits and transported on ice to the University of California Davis Medical Center Clinical Laboratory for processing within 4 h of collection. Red blood cell (RBC) folate was measured on fresh, never frozen, blood by an automated chemiluminescence assay [ACS 180; Chiron Diagnostics (now Bayer Diagnostics), Tarrytown, NY]. Plasma and serum were isolated and stored at  $-80^{\circ}\text{C}$  until analysis of vitamin B-12, homocysteine, and creatinine. Plasma vitamin B-12 was measured by radioassay (Quantaphase II; BioRad Diagnostics, Hercules, CA). Plasma homocysteine was measured by HPLC with postcolumn fluorescence detection (21). Serum creatinine was measured by standard spectrophotometric assay.

### Neuropsychological assessment instruments

Both global and specific subdomains of cognitive function were assessed by using 7 neuropsychological instruments and have been previously described (10, 22). Briefly, the 3MSE (23), which evaluates memory, orientation, attention, and language on a scale of 0–100, was used to determine global cognitive ability. The delayed recall test, based on a 15-point scale, was used to assess the ability to learn and recall verbal information (10, 22). The 3MSE and delayed recall instruments were administered to all but 10 subjects ( $n = 1779$ ). The other 5 neuropsychological instruments included object naming, picture association, verbal conceptual thinking, verbal attention span, and pattern recognition, each assessed on a 20-point scale (10, 22). These cognitive subdomain tests were administered to a subgroup of the total population ( $n = 537$ ) consisting of a random sample of 20% of the total population, as well as all subjects not in the 20% random sample who scored below the 20th percentile on either the 3MSE or the delayed-recall instruments. Subjects were given the choice of taking the neuropsychological tests in English or Spanish.

### Dementia diagnosis and assessment of depressive symptoms

Dementia diagnoses were established on the basis of neuropsychological test scores, mental status examination, the Informant Questionnaire on Cognitive Decline in the Elderly (24), medical history, and findings from a neurologic examination. Dementia was diagnosed based on *American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders, 3rd edition revised*, (25) criteria for dementia and the dementia criteria incorporated in the California Alzheimer Disease Diagnostic and Treatment Criteria for ischemic vascular dementia (26). Cognitive impairment with no dementia (CIND) was diagnosed if the person did not meet diagnostic criteria for dementia but had clinically significant impairment in  $\geq 1$  cognitive domain. Complete details of the diagnosis of dementia have been described in a previous publication (24). The Center for Epidemiologic Studies Depression (CES-D) scale was used to assess the presence of depressive symptoms on a scale of 0–60 points. A score of  $\geq 16$  is considered indicative of significant depressive symptoms (27).

### Demographic data

Age, sex, and the number of years of education were recorded for each subject. An acculturation score was determined by using the Acculturation Rating Scale for Mexican Americans-II (28), on a scale of 0–37 points.

### Statistical analysis

Multiple linear regression analyses were used to build 4 statistical models to describe the associations between RBC folate concentration (independent variable) and each of the cognitive function test instruments (dependent variables) before (model 1) and after adjustment for confounding by homocysteine alone (model 2), by homocysteine plus vitamin B-12 and creatinine (model 3), and by homocysteine plus vitamin B-12, creatinine, demographic (ie, age, sex, education, and acculturation), and depressive symptom (CES-D) variables (model 4). In addition, potential interactions between RBC folate and homocysteine on cognitive function scores were assessed by analysis of variance. In secondary analyses, odds ratios (ORs) were evaluated as indicators of the strength and direction of the relation between RBC folate and homocysteine (as independent continuous variables) and low 3MSE score ( $\leq 78$ ), low delayed recall score ( $\leq 6$ ), and dementia diagnosis. ORs with 95% CIs were determined by logistic regression in unadjusted models and in adjusted models controlling for potential confounding by age, sex, education, acculturation, vitamin B-12, creatinine, and CES-D score.

Because the values for RBC folate, plasma homocysteine, plasma vitamin B-12, and serum creatinine were not normally distributed (ie, there was tailing toward higher values), these variables were natural log-transformed before analysis. Statistical significance was defined for all analyses as  $P < 0.05$ . The statistical analyses were performed by using STATVIEW for MACINTOSH and WINDOWS (version 5.0.1; Abacus Concept, Berkeley, CA).

## RESULTS

Descriptive data for the SALSA population have been reported previously (10, 24), and only data relevant to the present



**TABLE 1**Description of Sacramento Area Latino Study on Aging (SALSA) population (total  $n = 1789$ )<sup>1</sup>

	$n^2$	Median (range)	Percentage abnormal (cutoff value)
			%
Sex (% female)	1789	58	—
Age (y)	1789	70 (60–101)	—
Education (y)	1778	6 (0–32)	—
Acculturation score (0–37)	1789	18 (0–37)	—
Homocysteine ( $\mu\text{mol/L}$ )	1642	9.8 (4.2–129)	17 ( $\geq 13$ )
RBC folate (ng/mL)	1501	488 (50–900)	0.9 ( $\leq 160$ )
Vitamin B-12 (pg/mL)	1545	416 (22–1968)	6.5 ( $\leq 200$ )
Creatinine (mg/dL)	1640		
Men	—	0.9 (0.4–10.2)	5.4 ( $> 1.4$ )
Women	—	0.7 (0.3–7.7)	6.5 ( $> 1.1$ )
3MSE score (0–100)	1779	86 (0–100)	22 ( $\leq 78$ )
Delayed recall score (0–15)	1779	9 (0–15)	25 ( $\leq 6$ )
CES-D score (0–60)	1663	6 (0–54)	25 ( $\geq 16$ )

<sup>1</sup> Data reported previously (10). RBC, red blood cell; 3MSE, Modified Mini-Mental State Examination (23); CES-D, Center for Epidemiological Studies Depression Scale (28).

<sup>2</sup> Sample sizes are the number of subjects with data available for each variable.

study are summarized in **Table 1**. We found that  $<1\%$  of the population had RBC folate concentrations below the cutoff for deficiency ( $\leq 160$  ng/mL), consistent with the government-mandated fortification of flour with folic acid. A large proportion (17%) had elevated plasma homocysteine ( $\geq 13$   $\mu\text{mol/L}$ ) despite folic acid fortification. Cognitive function scores indicative of significant cognitive impairment (3MSE  $\leq 78$ , delayed recall  $\leq 6$ ) were observed in 22–25% of the population. The prevalence of dementia was 3.9% ( $n = 70$ ), and an additional 3.8% ( $n = 68$ ) were diagnosed with CIND (24).

A series of 4 regression models that describe the associations between RBC folate and cognitive function scores is presented in **Table 2**. Before adjustment for confounding by homocysteine, vitamin B-12, creatinine, demographic and depressive symptom variables (Table 2, model 1), RBC folate was inversely correlated with all 7 cognitive function tests ( $P \leq 0.004$ ). The  $R^2$  values for these simple regressions indicate that RBC folate explains 1.6–

3.2% ( $R^2 = 0.016$ – $0.032$ ) of the variance in cognitive function scores within the sample. RBC folate remained inversely correlated with 6 cognitive function tests after the addition of homocysteine to the model (Table 2, model 2). The addition of vitamin B-12 and creatinine to the model had little influence on the associations between RBC folate and the cognitive function tests (Table 2, model 3). In contrast, the addition of the demographic variables (age, sex, education, acculturation) and CES-D score to the model significantly attenuated the associations between RBC folate and the cognitive function tests (Table 2, model 4), such that only the 3MSE and delayed recall tests remained significantly associated with RBC folate. The confounding variables that caused the greatest attenuation of the associations between RBC folate and the cognitive function tests were education and acculturation. For each analysis, the correlation coefficients for RBC folate were reduced in model 4 compared with models 1, 2, and 3, whereas  $R^2$  values in model 4 were 4- to 5-fold those in

**TABLE 2**Multiple linear regression models for red blood cell (RBC) folate (independent) compared with cognitive function scores (dependent)<sup>1</sup>

	Model 1			Model 2			Model 3			Model 4		
	$R^2$	Coefficient (SE)	$P$	$R^2$	Coefficient (SE)	$P$	$R^2$	Coefficient (SE)	$P$	$R^2$	Coefficient (SE)	$P$
3MSE	0.018	5.63 (1.08)	$< 0.001$	0.041	3.89 (1.09)	$< 0.001$	0.069	4.47 (1.11)	$< 0.001$	0.353	2.16 (0.77)	0.005
Delayed recall	0.016	1.12 (0.023)	$< 0.001$	0.061	0.62 (0.23)	0.008	0.076	0.87 (0.24)	$< 0.001$	0.293	0.55 (0.21)	0.007
Object naming	0.024	1.91 (0.57)	$< 0.001$	0.046	1.32 (0.60)	0.028	0.111	1.37 (0.59)	0.021	0.506	0.42 (0.45)	NS
Picture association	0.026	2.20 (0.63)	$< 0.001$	0.053	1.49 (0.65)	0.022	0.114	1.36 (0.65)	0.037	0.562	0.32 (0.47)	NS
Verbal conceptual thinking	0.032	2.23 (0.57)	$< 0.001$	0.057	1.59 (0.59)	0.008	0.102	1.67 (0.60)	0.005	0.467	0.43 (0.46)	NS
Verbal attention	0.027	2.01 (0.56)	$< 0.001$	0.040	1.56 (0.59)	0.008	0.077	1.48 (0.60)	0.015	0.344	0.46 (0.54)	NS
Pattern recognition	0.018	1.94 (0.67)	0.004	0.047	1.16 (0.70)	NS	0.121	1.08 (0.68)	NS	0.455	−0.12 (0.56)	NS

<sup>1</sup> Multiple linear regression models are described as follows: model 1, ln (RBC folate) alone; model 2, model 1 + ln (homocysteine); model 3, model 2 + ln (vitamin B-12) + ln (creatinine); and model 4, model 3 + age + sex + education + acculturation + Center for Epidemiologic Studies Depression score.  $R^2$  values are those for entire model including ln (RBC folate) and covariates. Coefficients (SE) and  $P$  values are for ln (RBC folate) within each model. Sample sizes for the Modified Mini-Mental State Examination (3MSE) and delayed recall tests varied from  $n = 1476$  (model 1) to  $n = 1356$  (model 4). Sample sizes for the object naming, picture association, verbal conceptual thinking, verbal attention, and pattern recognition tests varied from  $n = 461$  (model 1) to  $n = 411$  (model 4).

**TABLE 3**

Odds ratios (ORs) for low Modified Mini-Mental State Examination (3MSE) score ( $\leq 78$ ), low delayed recall score ( $\leq 6$ ), and dementia diagnosis by natural log of red blood cell (RBC) folate<sup>1</sup>

	Model 1			Model 2			Model 3		
	<i>n</i>	OR (95% CI)	<i>P</i>	<i>n</i>	OR (95% CI)	<i>P</i>	<i>n</i>	OR (95% CI)	<i>P</i>
3MSE score $\leq 78$	1474	0.40 (0.28, 0.58)	< 0.001	1473	0.49 (0.34, 0.71)	< 0.001	1356	0.51 (0.32, 0.83)	0.006
Delayed recall score $\leq 6$	1476	0.59 (0.40, 0.78)	< 0.001	1475	0.72 (0.51, 1.02)	NS	1359	0.67 (0.44, 1.01)	NS
Dementia diagnosis <sup>2</sup>	1501	0.38 (0.20, 0.73)	0.003	1500	0.49 (0.25, 0.95)	0.036	1374	0.28 (0.11, 0.72)	0.008

<sup>1</sup> ORs (95% CI) were determined by logistic regression analyses and are per increment of 1 unit in the natural log of RBC folate. The logistic regression models are described as follows: model 1, ln (RBC folate) alone; model 2, model 1 + ln (homocysteine); and model 3, model 2 + ln (vitamin B-12) + ln (creatinine) + age + sex + education + acculturation + Center for Epidemiologic Studies Depression score. Sample sizes are less in the adjusted models because of missing values among the covariates.

<sup>2</sup> Subjects with dementia were compared with normal subjects (no cognitive impairment, no dementia). Subjects with cognitive impairment but no dementia (CIND) were included as a third diagnostic group in the logistic regression. No significant ORs for CIND by ln (RBC folate) were observed (data not shown).

model 3. These  $R^2$  values indicate that the demographic and CES-D variables explain a much higher proportion of the variance in cognitive function scores than do the biochemical variables (folate, homocysteine, vitamin B-12, and creatinine). In addition, as indicated by analysis of variance, the associations among RBC folate and 3MSE and delayed recall scores were not modified by the interaction of RBC folate with homocysteine, and the interaction among these factors was not significant (data not shown).

In secondary analyses, ORs were evaluated as indicators of the strength and direction of the relations among RBC folate (as a continuous variable) and 3MSE, delayed recall, and dementia diagnosis. These relations were evaluated by logistic regression in unadjusted models and in adjusted models controlling for potential confounding by homocysteine alone, and then homocysteine plus age, sex, education, acculturation, vitamin B-12, creatinine, and CES-D score. In the unadjusted models (Table 3, model 1), ORs for low 3MSE score ( $\leq 78$ ), low delayed recall score ( $\leq 6$ ), and dementia diagnosis were below unity and were statistically significant ( $P \leq 0.003$ ), indicating that the relative risks (RRs) of cognitive impairment and dementia decreased with increasing RBC folate concentration. After adjustment for homocysteine alone (Table 3, model 2) and for homocysteine plus the other confounding variables (Table 3, model 3), the ORs for low 3MSE score and dementia diagnosis remained significant

( $P \leq 0.036$ ), whereas the OR for low delayed recall score was not significant. No significant association was observed between RBC folate and diagnosis of CIND (data not shown).

We also evaluated ORs as indicators of the strength and direction of the relations among homocysteine (as a continuous variable) and 3MSE, delayed recall, and dementia diagnosis. As was the case for RBC folate, these relations were evaluated by logistic regression in unadjusted models and in adjusted models controlling for potential confounding by RBC folate alone, and then RBC folate plus the other confounding variables. In the unadjusted models (Table 4, model 1) and in the models adjusted for RBC folate alone (Table 4, model 2), ORs for low 3MSE score, low delayed recall score, and dementia diagnosis were above unity and were statistically significant ( $P \leq 0.002$ ), indicating that the RRs of cognitive impairment and dementia increased with elevated homocysteine concentration. However, after controlling for RBC folate plus the other confounding variables (Table 4, model 3), the ORs for low cognitive function scores and dementia diagnosis were not significant. As for low cognitive function scores and dementia diagnosis, the ORs for CIND diagnosis were significant for the unadjusted model and the model adjusted for RBC folate alone but not for the model adjusted for RBC folate plus all the other confounding variables (data not shown).

**TABLE 4**

Odds ratios (ORs) for low Modified Mini-Mental State Examination (3MSE) score ( $\leq 78$ ), low delayed recall score ( $\leq 6$ ), and dementia diagnosis by natural log of homocysteine<sup>1</sup>

	Model 1			Model 2			Model 3		
	<i>n</i>	OR (95% CI)	<i>P</i>	<i>n</i>	OR (95% CI)	<i>P</i>	<i>n</i>	OR (95% CI)	<i>P</i>
3MSE score $\leq 78$	1614	2.53 (1.80, 3.55)	< 0.001	1473	2.10 (1.46, 3.03)	< 0.001	1356	0.99 (0.50, 1.96)	NS
Delayed recall score $\leq 6$	1609	3.00 (2.15, 4.18)	< 0.001	1475	2.96 (2.06, 4.27)	< 0.001	1359	1.12 (0.64, 1.97)	NS
Dementia diagnosis <sup>2</sup>	1642	2.95 (1.70, 5.12)	< 0.001	1500	2.54 (1.39, 4.65)	0.002	1374	2.70 (0.62, 11.81)	NS

<sup>1</sup> ORs (95% CI) were determined by logistic regression analyses and are per increment of 1 unit in the natural log of homocysteine. The logistic regression models are described as follows: model 1, ln (homocysteine) alone; model 2, model 1 + ln (RBC folate); and model 3, model 2 + ln (vitamin B-12) + ln (creatinine) + age + sex + education + acculturation + Center for Epidemiologic Studies Depression score. Sample sizes are less in the adjusted models because of missing values among the covariates.

<sup>2</sup> Subjects with dementia were compared with normal subjects (no cognitive impairment, no dementia). Subjects with cognitive impairment but no dementia (CIND) were included as a third diagnostic group in the logistic regression. ORs for CIND by ln (homocysteine) were significant in models 1 and 2 ( $P \leq 0.01$ ) but not in model 3 (data not shown).



## DISCUSSION

The main finding of this study is that RBC folate is directly associated with cognitive function scores and inversely associated with dementia in elderly Latinos despite folic acid fortification. The data indicate that RBC folate is associated with 3MSE and delayed recall scores after controlling for confounding by homocysteine, as well as other biochemical (vitamin B-12, creatinine) and demographic (age, sex, education, acculturation) variables and depressive symptom score. Furthermore, RRs of cognitive impairment and dementia decreased with increasing RBC folate concentration. These findings are consistent with and extend our previous report of data from this cohort in which the mean RBC folate was significantly lower in subjects with dementia than in healthy subjects without dementia or CIND (29). In addition, subjects with dementia had a higher prevalence of RBC folate below the cutoff value for deficiency ( $<160$  ng/mL) than did the cognitively normal subjects (29).

A limitation of this study is that the cross-sectional design precludes determination of cause and effect. It is possible that cognitive impairment and dementia could negatively affect dietary intake and thus folate status. Arguing against this possibility is the very low prevalence of folate deficiency ( $<1\%$ ), consistent with a population that is receiving the benefits of folic acid fortification. Of interest will be how baseline folate status affects incident cognitive dysfunction and dementia in the SALSA population. The possible effect of confounding is another potential limitation of the study. The confounding factors that we controlled for in the statistical analyses were chosen a priori to be consistent with our previous publication on homocysteine and cognitive function in this population (10). However, it is possible that other confounding factors, such as vascular disease, medications, and lifestyle habits (eg, smoking, alcohol consumption), could have influenced the results.

In our previous publication in the SALSA study on homocysteine and cognitive function (10), we reported that homocysteine was inversely associated with both RBC folate and several cognitive function tests, including 3MSE, picture association, verbal attention, and pattern recognition. The associations between homocysteine and the cognitive function tests remained significant after controlling for confounding by RBC folate. In contrast, the present analysis indicates that RBC folate is associated with 3MSE and delayed recall scores but not with picture association, verbal attention, and pattern recognition. In addition, homocysteine was not associated with a diagnosis of dementia. These results are consistent with the findings of Riggs et al (6) who found that folate status was more strongly correlated than plasma homocysteine with delayed recall scores in the Boston Normative Aging study (men aged 54–81 y;  $n = 70$ ). These findings suggest that despite that folate is a determinant of homocysteine, low folate status and hyperhomocysteinemia may have differential effects on discrete cognitive domains and discrete regions of the brain.

This raises the issue of mechanisms by which folate and homocysteine may affect brain function. Hyperhomocysteinemia is a risk factor for cerebrovascular disease (1) and has been shown to predict incident dementia in the Framingham study cohort (30). More basic research has indicated that homocysteine induces excitotoxic effects in brain through increased glutamate receptor activation (31). Folate deficiency may affect the brain

by reducing synthesis of SAM and thus inhibiting SAM-dependent methylation reactions. Such reactions include the synthesis and catabolism of many neurotransmitters, including dopamine, norepinephrine, adrenaline, and serotonin. Serotonin is of particular interest because deficiency of this neurotransmitter is associated with depression. Folate deficiency is also associated with depression (32), and we have previously shown that low plasma folate is associated with elevated depressive symptoms in women, but not men, in the SALSA study (33). Moreover, oral SAM has antidepressant effects (34). Because depression is a strong determinant of cognitive function in older adults, the association between low folate status and cognitive function may be related to SAM, serotonin synthesis, and depression. However, in the present study, RBC folate remained associated with cognitive function and dementia after controlling for depressive symptoms. These observations suggest that depression does not explain the observed associations between RBC folate and cognitive function. The issue of how low folate status and hyperhomocysteinemia influence brain function remains an important area of investigation.

The results of the present study are consistent with previous studies performed before folic acid fortification in the United States or in other countries without folic acid fortification (6, 11–16). Particularly relevant is the New Mexico Elder Health Survey, a cohort study of Hispanic and non-Hispanic white elderly ( $\geq 65$  y;  $n = 783$ ) (15). Subjects with serum folate  $< 11.1$  nmol/L had lower mean scores for global cognitive function and several tests of memory than did subjects with serum folate  $\geq 11.1$  nmol/L. Quadri et al (11) assessed a cohort of elderly subjects ( $\geq 60$  y) seen at a memory clinic in Switzerland ( $n = 228$ ). Subjects with mild cognitive impairment ( $n = 81$ ) or dementia ( $n = 92$ ) had significantly lower mean serum folate concentrations than control subjects without cognitive impairment ( $n = 55$ ). Furthermore, those subjects in the lowest tertile of serum folate ( $< 13.5$  nmol/L) had an adjusted OR of 3.8 (95% CI: 1.3, 11.2) for dementia compared with the highest tertile of serum folate ( $> 19.5$  nmol/L) ( $P = 0.018$ ). In a multicenter Canadian study of institutionalized and noninstitutionalized elderly ( $\geq 65$  y;  $n = 1171$ ), Ebly et al (14) found that there was a higher percentage of subjects with dementia in the lowest quartile of serum folate ( $\leq 10$  nmol/L) compared with the highest quartile ( $> 14$  nmol/L) ( $P < 0.0001$ , chi-square test). Moreover, the mean 3MSE score for the subjects in the lowest quartile of serum folate was significantly lower than for subjects in the highest quartile (62.9 compared with 70.4, respectively;  $P = 0.003$ ).

The finding that low folate status is associated with poor cognitive function and dementia, despite folic acid fortification, may lead some to conclude that more folic acid should be added to the food supply or, alternatively, that older adults should be encouraged to take folic acid supplements to reduce the risk of cognitive impairment and dementia. However, we caution that this conclusion may be premature. Cognitive impairment and dementia are pathologic processes that typically develop over years or even decades. Although this cross-sectional study was conducted in a population currently exposed to folic acid fortification, this exposure began within 1.5 y before the collection of blood samples and assessments of cognitive function. Folate status may have influenced the development of cognitive impairment and dementia in the years before folic acid fortification. The relation between current folate status in this population and prefortification status is unknown, although fortification has certainly

shifted the distribution of RBC folate concentrations to higher values. It can be hypothesized that individuals who had low (ie, deficient) folate status before fortification remain in the low end of the current distribution of folate concentrations, even though most of these subjects are not currently folate deficient. If this is the case, then it would be expected that those subjects in the low end of the current distribution would have a greater RR of having low cognitive function scores and dementia than those subjects in the high end of the distribution, as was observed. Also, cognitive impairment caused by folate deficiency may become irreversible if the deficiency is prolonged. Therefore, folate status may improve with fortification but have no effect on cognitive function. What remains to be determined is the effect of the current amount of folic acid fortification on incident cognitive impairment and dementia in longitudinal studies of older adults.

Additionally, the elderly population in general, as well as the particular demographic group in whom this study was conducted (35), has a high prevalence of vitamin B-12 deficiency. The current high intake of folic acid by the population as a whole, and the elderly in particular, may be masking vitamin B-12 deficiency by attenuating hematologic evidence of the deficiency, that is, normalizing mean corpuscular volume and hemoglobin concentration. Therefore, further increasing the amount of folic acid in the food supply or recommending supplements may be premature.



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